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ENCAPSULATED NONGAS-FORMING BACILLI

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In 1882, Friedländer found in the sputum of pneumonia patients an encapsulated bacillus to which he gave the name Bacillus pneumoniae. Since that time our knowledge concerning organisms characterized by capsule formation has been extended until at present we have three well-defined types of encapsulated bacilli, namely, B. pneumoniae, B. acidi-lactici, and B. lactis-aerogenes. There is still much uncertainty concerning the characteristics of the subgroups of these organisms. Practically all investigators who have attempted to classify these bacilli have reported irregularities in the cultural reactions. Each worker has presented one or more strains which failed to show cultural reactions corresponding throughout with any member of the fixed groups.

With few exceptions the organisms of this class hitherto described produce acid and gas on one or more of the various carbohydrates. Thus, Strong[1] and Coulter[2] dealt only with the gas-forming types from numerous sources. Herrold and Culver[3] reported 16 strains of gas-forming encapsulated bacilli in a series of 86 cases of renal infections. Meyer and Hinman[4] reported an encapsulated nongas-forming hemoglobinophilic bacillus from a patient suffering from double hydronephrosis. Perkins[5] working with cultures from different localities divided his bacilli into 3 groups: (1) those that fermented all the carbohydrates used; (2) those that fermented all but one or two carbohydrates used; (3) those that fermented none of the carbohydrates. In the last group he placed B. rhinoschleromae, an organism identified by him as Hopkins culture No. 5, and an organism which he had received from Král's Laboratory. The last culture fermented the carbohydrates after passage through an animal. This observation leads one to question whether the author actually recovered the same organisms which he injected, or one of the other encapsulated organisms which are known to inhabit the intestinal tract of both man and animals.

A review of the literature has revealed no reports of encapsulated nongas-forming bacilli other than those cited. Recently, we have

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[1] Centralbl. f. Bakteriol., I, 1899, 25, p. 49.

[2] Jour. Exper. Med., 1917, 26, p. 763.

[3] Jour. Infect. Dis., 1919, 24, p. 114, and Jour. Am. Med. Assn., 1918, 70, p. 1444.

[4] Ibid., 1920, 27, p. 72.

[5] Ibid., 1904, 1, p. 241.

isolated from different sources three organisms of this description. This paper is a report of the morphologic, cultural, and immunologic characteristics of these organisms.

Culture No. 1 was isolated from a white man, aged 44, who had always enjoyed good health until a short time before he came to the genito-urinary clinic for treatment. He gave a history of having had the usual symptoms of an acute cystitis. These acute symptoms had subsided before the patient applied for treatment. He was somewhat annoyed by the continuation of the cloudiness of the urine and by the offensive odor given off by the urine at the time of micturition.

On cystoscopic examination the patient presented a chronic cystitis. A specimen of urine removed from the bladder by catheter was extremely turbid and had a foul odor similar to that produced by decomposing protein material. Examination showed a heavy trace of albumin, many pus cells, a few red blood cells, and many nonmotile bacteria. Culture of this specimen revealed a small gram-negative, encapsulated, nongas-forming, aerobic bacillus. Cultures of urine from this patient at three different times during the next four months showed the same organism.

Culture No. 2 was isolated from a white woman, aged 30 years, who entered the hospital complaining of periodic attacks of abdominal pain. The trouble dated back to her first pregnancy about 8 years ago. The patient said that she had had attacks of severe abdominal pain at intervals of approximately 6 months, since the onset of the trouble; that there was pain on urination; and that during these attacks the urine was always bloody. There was a contracted urethral meatus which required mechanical dilatation before a cystoscopic examination could be satisfactorily accomplished. The findings were a pyelitis of the left kidney and a normal bladder. Specimens of urine were obtained from the bladder and left ureter. They showed a moderate amount of albumin, many pus cells, and a few red blood cells. Cultures of the urine from the left ureter and the bladder contained a small gram-negative, encapsulated, nongas-forming, aerobic bacillus.

Culture No. 3 was obtained at necropsy from the left pleural cavity of a white woman, aged 30 years, who had been complaining for 12 years or more of "kidney trouble." One year before death the urine was found heavily loaded with albumin, and she was refused an operation which previously had been advised. Several months later, she was admitted to the medical service of the Allegheny General Hospital, where the diagnosis of lobar pneumonia, endocarditis, and uremia was made. Just prior to admission she developed subcutaneous ecchymoses and considerable bleeding from the nose and gums. She died 8 days after admission. Necropsy revealed: acute and chronic interstitial and parenchymatous nephritis; chronic endocarditis, myocarditis, and pericarditis; arteriosclerosis; bronchopneumonia, acute and chronic pleurisy with effusion; subconjunctival and palpebral ecchymosis; healed tubercles of the lung, liver and spleen; and anasarca. The left pleural cavity contained a yellow flaky fluid from which was cultivated streptococcus hemolyticus and a small gram-negative, encapsulated, nongas-forming, aerobic bacillus similar to those already described.

DESCRIPTION OF THE ORGANISM

The organism under discussion is a gram-negative, nonspore-bearing, encapsulated, nonmotile rod which varies in length from 1 to 2 mikrons—and in diameter from .3 to .5 mikrons, in general being a little smaller than the typhoid bacillus. The capsules differ somewhat in their size and staining characteristics. In the first culture they are demonstrated easily from any type of medium, while in the other two cultures they are demonstrated only when grown in milk. The relative size of the capsules compares with the thick capsules of the B. lactis-aerogenes type somewhat as the capsules of pneumococci types 1, 2 and 4 compare with the capsules of type 3; that is, the capsules of these organisms stain more faintly, appear to be smaller, and the bacilli occupy a relatively smaller amount of the capsule than those of B. lactis-aerogenes. On gelatin and agar the colonies vary from thin translucent coli-like colonies of medium size to large thick slimy mucoid aerogenes-like colonies. They grow best at 37 C. and more slowly at 20 C. The cultural reactions of the organisms obtained from the 3 sources described correspond throughout with few exceptions. In table 1 the cultural characteristics of each, observed over a period of 3 weeks, are recorded. It shows their similarity and the slight points of variation which exist between them.

If the results recorded are examined in relation to the types of carbohydrates used, several interesting points will be noted.

1. Monosaccharids:		
(*a*) Pentoses	arabinose rhamnose	acid, no gas
	xylose	no acid, no gas
(*b*) Hexoses	dextrose mannose galactose levulose	acid, no gas
	inosite	no acid, no gas
2. Disaccharids:		
	maltose	acid, no gas
	lactose sucrose	no acid, no gas
3. Trisaccharids:		
	raffinose	no acid, no gas
4. Polysaccharids:		
	dextrin	acid, no gas
	inulin salicin	no acid, no gas
5. Alcohols:		
	mannitol dulcitol sorbitol	acid, no gas
6. Glycerol:		
	glycerol	no acid, no gas

TABLE 1

Cultural Characteristics *

Cultures	Dextrose	Levulose	Galactose	Maltose	Mannitol	Xylose	Arabinose	Rhamnose	Sorbitol	Dulcitol	Lactose	Salicin	Sucrose	Raffinose	Inosite	Dextrin	Mannose	Inulin	Glycerol	Milk	Gelatin	Indol	Lead Acetate	Motility	Pathogenic
Culture 1:																									
First day........	ac	ac	ac	ac	ac	ac	ac	t	ac	ac	t	t	t	t	t	ac	ac	t	ac	nc	—	+	—	—	+
Third day........	ac	ac	ac	ac	ac	ac	ac	ac	ac	ac	t	t	t	t	t	ac	ac	t	ac	nc	—	+	—	—	+
Fifth day........	ac	dec	ac	ac	ac	ac	ac	ac	ac	ac	t	t	t	t	t	dec	ac	t	ac	dec	—	+	—	—	+
Tenth day.......	ac	dec	ac	ac	ac	ac	ac	ac	ac	ac	t	t	t	t	t	dec	ac	t	ac	str alk	—	+	—	—	+
Fourteenth day..	ac	dec	ac	ac	ac	ac	ac	ac	ac	ac	t	t	t	t	t	dec	ac	t	dec	alk. sl pep	—	+	—	—	+
Twenty-first day	ac	dec	ac	ac	ac	ac	ac	ac	ac	ac	t	t	t	t	t	dec	ac	t	dec	alk, pep	—	+	—	—	+
Culture 2:																									
First day........	ac	ac	ac	ac	ac	ac	ac	t	ac	t	t	t	t	t	t	ac	ac	t	ac	nc	—	+	—	—	+
Third day........	ac	ac	ac	ac	ac	ac	ac	t	ac	ac	t	t	t	t	t	ac	ac	t	ac	nc	—	+	—	—	+
Fifth day........	ac	ac	ac	ac	ac	ac	ac	ac	ac	ac	t	t	t	t	t	ac	ac	t	ac	dec	—	+	—	—	+
Seventh day.....	ac	ac	ac	ac	ac	ac	ac	ac	ac	ac	t	t	t	t	t	ac	ac	t	ac	alk	—	+	—	—	+
Tenth day.......	ac	ac	ac	dec	ac	ac	ac	ac	ac	ac	t	t	t	t	t	dec	ac	t	ac	str alk	—	+	—	—	+
Fourteenth day..	ac	ac	ac	dec	ac	ac	ac	dec	ac	dec	t	t	t	t	t	dec	ac	t	ac	alk, sl pep	—	+	—	—	+
Twenty-first day	ac	ac	ac	dec	ac	ac	ac	dec	ac	dec	t	t	t	t	t	dec	ac	t	dec	alk, pep	—	+	—	—	+
Culture 3:																									
First day........	ac	ac	ac	ac	ac	ac	ac	t	ac	t	t	t	t	t	t	ac	ac	t	ac	nc	—	+	—	—	+
Third day........	ac	ac	ac	ac	ac	ac	ac	ac	ac	ac	t	t	t	t	t	ac	ac	t	ac	nc	—	+	—	—	+
Fifth day........	ac	ac	ac	ac	ac	ac	ac	ac	ac	ac	t	t	t	t	t	ac	ac	t	ac	dec	—	+	—	—	+
Tenth day.......	ac	ac	ac	dec	ac	ac	ac	ac	ac	ac	t	t	t	t	t	dec	ac	t	ac	str alk	—	+	—	—	+
Fourteenth day..	ac	ac	ac	dec	ac	ac	ac	ac	ac	ac	t	t	t	t	t	dec	ac	t	ac	alk, sl pep	—	+	—	—	+
Twenty-first day	ac	ac	ac	dec	ac	ac	ac	dec	ac	dec	t	t	t	t	t	dec	ac	t	ac	alk, pep	—	+	—	—	+

ac = acid; alk = alkaline; sl = slightly; str = strongly; pep = peptonization; dec = decolorized; t = turbid; nc = no change; + = positive; — = negative.

* In order to be certain that the carbohydrates were reacting properly, we cultivated a series of control organisms parallel with the unknown strains. Those used as controls were: B. lactis-aerogenes, B. coli-communis, B. coli-communior, B. typhosus, B. paratyphosus A and B, and B. dysenteriae; and each strain gave its typical reaction on each of the carbohydrates.

It is to be noted that the organisms show no particular preference for any group of carbohydrates. They produce acid on 2 pentoses, 4 hexoses, 1 disaccharid, 1 polysaccharid and 3 alcohols. They fail to produce acid on 1 pentose, 1 hexose, 2 disaccharids, 1 trisaccharid, 2 polysaccharids, and 1 glycerol.

The production of indol is constant with the three cultures.

The reactions on litmus milk are the most striking and characteristic cultural findings. In the first 3 days there is no change. On the third day the medium is decolorized or develops a chalky appearance which lasts until the sixth day. At this time a slight trace of alkalinity develops; and on the seventh day a definite alkalinity is noted which gradually increases to a maximum reached on the tenth day. Coagulation does not occur. The casein of the milk is peptonized after the appearance of the alkaline reaction, the medium gradually loses its opacity and becomes a transparent deep blue. It is noted that when the tubes of litmus milk are inoculated and immediately sealed, or sealed within 24 hours, the change in the litmus milk proceeds only to the stage of decolorization, and that neither the peptonization nor secondary alkalinity develops. This suggests that perhaps all of the available oxygen in the tube has been consumed and that there is not a sufficient amount remaining to permit the complete reaction described to take place as it does when the tubes are not sealed.

It will be noted on examination of table 1 that the cultural reactions of these bacilli are essentially the same as those of B. dysenteriae (Flexner). The Flexner organism, however, does not possess a capsule and is less alkaline on litmus milk.

PATHOGENICITY

Each of the 3 strains when first isolated was highly pathogenic for guinea-pigs and rabbits, but has become less pathogenic after prolonged artificial cultivation. When first isolated 1 cc of a 24-hour broth culture given intravenously, killed, in 30 minutes, a rabbit weighing 2 kilograms; but when given intraperitoneally, it required 24 hours to kill a rabbit of similar weight. Necropsy on these animals showed an acute diffuse peritonitis similar to that produced by B. coli. Similar reactions were obtained with guinea-pigs. It was also found that 1 cc of the 24 to 48 hour broth culture when freed from the bacilli, contained sufficient toxic material to produce a severe reaction in rabbits almost instantly.

IMMUNOLOGIC REACTIONS

The serum from the first patient agglutinated the organism isolated from him, in dilutions varying from 1/10 to 1/160, while the serum from the second patient did not agglutinate either the organism obtained from her or that obtained from the first patient. No tests were made with the serum of the third patient as the culture was obtained at necropsy.

Rabbits were immunized against each of the 3 cultures. Immunization was started by injecting intravenously small doses of the organisms suspended in saline and heated for 30 minutes at 56 C. For subsequent injections the time of heating was gradually decreased until it was possible to give living organisms intravenously without apparent damage to the animals.

TABLE 2

AGGLUTININ TITERS

Bacilli	Antiserums				
	Anti-serum 1	Anti-serum 2	Anti-serum 3	Anti-dysentery (Flexner) Serum	Antiserums for Typhoid, Paratyphoid, Dysentery (Shiga, Hiss-Y, Rosen), Enteritidis Bacilli
Bacillus 1	400	0	1600	50	0
Bacillus 2	50	50	50	50	0
Bacillus 3	100	100	100	50	0
Typhoid, paratyphoid, dysentery (Flexner, Shiga), pseudodysentery, lactis aerogenes bacilli	0	0	0		

The figures give the highest active dilution of the antiserum in each case.

Ten days after the last injection the animals were bled and the serum of each was tested against the three strains in question, against B. typhosus, B. paratyphosus A and B, B. dysenteriae (Flexner), B. dysenteriae (Shiga), a pseudodysentery bacillus, and B. lactis-aerogenes.

Table 2 shows that the animals immunized against 2 of the organisms developed strong agglutinating power for their homologous strains and somewhat less agglutinating power for the other 2.

In order to compare fully these organisms serologically with the typhoid-dysentery group, emulsions of the three types of bacilli were tested with serum against the following organisms: B. typhosus, B. paratyphosus A and B, B. dysenteriae (Shiga), B. dysenteriae (Flexner), B. dysenteriae (Hiss-Y), B. dysenteriae (Rosen), and B. enteritidis (Gartner).

It is interesting to note (table 2) that the only serum which agglutinates these bacilli at all is that against B. dysenteriae (Flexner). These reactions added to those on the carbohydrates indicate that there is a close relationship between the encapsulated bacilli described in this report and the B. dysenteriae (Flexner).

DISCUSSION

In reviewing the literature on encapsulated gram-negative bacilli, we realize the need of a more thorough and systematic study of this group which Winslow, Kligler, and Rothberg[6] have designated as "an extraordinarily variable group." These authors have given an admirable classification of the colon-typhoid group of bacteria, but seem to have been content to pass by the encapsulated group with a short description of B. aerogenes (Escherich) and a few words about the irregular forms of this group.

Other authors,[7] make more or less limited reports, not taking up in detail the common irregularities which one so frequently encounters in the routine work of a large hospital. We believe that bacteriologists are beginning to realize the need of more detailed knowledge of this group of organisms, particularly since they are so frequently found in infections of the genitro-urinary tract. On account of their close resemblance to the colon bacilli in morphology, staining and cultural reactions, it is possible that they are not infrequently mistaken for colon bacilli.

If such work as that of Winslow, Kligler, and Rothberg[6] can be extended to other groups of organisms, we shall be able to avoid much of the confusion that has arisen and continues to arise among the bacteria which are so variable.

It is interesting to note the reports of encapsulated forms of B. paratyphosus B, and B. dysenteriae by Fletcher[8] which the author states were made without a complete investigation of the organisms, because the military service made it impossible for him to give sufficient time to complete the work properly. He also states that from peptone water he could obtain both encapsulated and nonencapsulated forms. We would suggest, therefore, that probably the author was not dealing with a pure culture of B. paratyphosus B, or B. dysenteriae, but that he probably had a mixed culture of B. paratyphosus B and a slow lactose fermenting B. acidi-lactici which is also encapsulated. We have isolated one culture of this type which did not ferment lactose until the fourteenth day.

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[6] Jour. Bacteriol., 1919, 4, p. 429.

[7] Levine: Jour. Am. Public Health Assn., 1917, 7, p. 784. Fitzgerald: Jour. Infect. Dis., 1914, 15, p. 268

[8] Lancet, 1918, 2, p. 102.

The organisms we describe are apparently closely related to the organism described by Meyer and Hinman, and yet there are several points of difference. Their organism was hemoglobinophilic, grew in small mucoid colonies, and when smears were made from the growth with a platinum loop, the whole colony was frequently removed. Our bacilli, on the other hand, are not hemoglobinophilic, they grow readily on all ordinary culture mediums and the colonies resemble under some conditions, typical B. coli colonies, and under others, typical B. lactis-aerogenes colonies. It is practically impossible to remove the whole colony at one time with the platinum loop. The cultural reactions, however, are almost identical with those obtained by Meyer and Hinman.

If the bacilli which we describe here belong in the colon-typhoid group of bacilli, they should be placed in group 2, according to the classification of Winslow, Kligler, and Rothberg, along with B dysenteriae (Flexner). There is a close relationship culturally and an apparent one immunologically between the two. If they do not belong in the colon-typhoid group of bacilli, we believe that B. pneumoniae, B. acidi-lactici, and B. lactis-aerogenes should also be removed from that group and that all of the encapsulated bacilli should be placed in a group separate and distinct from the colon-typhoid group. The organism we describe seems to occupy a position among the encapsulated bacilli similar to that occupied by the B. dysenteriae (Flexner) in the typhoid-paratyphoid-dysentery group.

SUMMARY

A previously unrecognized organism, a small gram-negative, encapsulated, nongas-forming, aerobic bacillus was found in three patients, each of whom was suffering from some pathologic condition of the genito-urinary tract. It was not possible to determine whether the organism was the primary etiologic factor in producing the lesions or a secondary invader.